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Influence of metal compounds on Fulvic Acid/ Molybdenium Blue reactive phosphate associations

By Christian Steinberg and G. Friedrich Baltes, Munich

With 4 figures in the text

Abstract

Separation of fulvic acid/molybdenium blue reactive phosphorus (MRP) compounds from raised peat bog water using gelpermeation chromatography (Sephadex G-25) revealed a bimodal distribution pattern of phosphates: low molecular weight (lmwt) and high molecular weight (hmwt) MRP. Hmwt MRP seems to be sensitive to UV-radiation. The additions of iron ions (up to 1.5 mg/l Fe) and of manganese in low concentrations caused decreases of lmwt MRP and simultaneous increases of hmwt MRP by formation of colloids. Higher amount additions of manganese ions (up to 5.0 mg/l Mn), as well as each addition of cadmium ions, led to decreases of hmwt MRP and increases of lmwt MRP, simultaneously. The latter effect is most likely due to catalytic cleavage of hmwt MRP by the added metals.

Introduction

In natural waters of different sources, a bimodal distribution of phosphates, which may be determined by common molybdenium blue methods without previous hydrolysis, can often be found by separating dissolved organic water constituents, mainly fulvic acids (see e.g. Francko & Heath, 1979, 1982, 1983; Downes & Paerl, 1978; White & Payne, 1980; Steinberg & Schrimpf, 1980). In the main, the achieved apparently low molecular weight (≙ lmwt) *gel*permeation chromatography fractions consist of phosphoric acid esters and orthophosphate, the apparently high molecular weight (≙ hmwt) GPC fraction resist enzymatic attack by phosphatases (Francko & Heath, 1979). Already in 1935, Ohle described this fact as a hypothesis: "The greatest moieties of the most significant nutrients in calcium poor brown waters are bound by adsorption to organic substances... These nutrients, e.g. phosphate, wich are chained into organic micella are even resistant to chemical agents and definitely do not diffuse through semipermeable cellular membranes." This obviously unavailability of hmwt phosphates has been proved in the meantime (Francko & Heath, 1979; Stewart & Wetzel, 1981). According to recent findings, UV-light cleaves most of the associations between phosphate and hmwt fulvic acids (Francko & Heath, 1979).

In bog waters, great moieties of the organically bound phosphates are found in the hmwt fraction. According to recent findings, iron in its ferric state takes significantly part in forming these associations (FRANCKO & HEATH, 1982). Own experiments (STEINBERG & STABEL, 1978) showed that more than 90% of the dissolved iron ($<0.6\,\mu m$) were found in the hmwt carbon fraction of raised peat bog water.

In the present study, the influence of metals upon forming hmwt molybdenium blue reactive phosphates (MRP) was tested with water from the Latschensee, a raised peat bog. As cited reports stress the importance of the oxidation status of the metals for this process, we studied the influence of iron and manganese, both redox variable metals. Redox-independent cadmium served as basis of comparison.

Materials and methods

The water tested was collected from the Latschensee, a raised peat bog. This small lake is located in the catchment area of the Frauenau drinking water reservoir in the Schachten district, Bayerischer Wald, near the ČSSR-border. Fulvic and humic acids of this lake derived mainly from the decomposition of *Sphagnum* mosses.

The water had a pH-value of 3.85, a Fe-concentration of $70\,\mu g/l$, a Mn-concentration of $116\,\mu g/l$ and a Cd-concentration of less than $1\,\mu g/l$ (determinations by means of atomic absorption spectroscopy). The water was filtered through membranes (pore size $<0.45\,\mu m$) at slight low-pressure.

A Sephadex G-25 gel by Pharmacia was used for GPC. To avoid adsorption and retardation of organic compounds by the matrix as far as possible, the water was titrated with $^1/_{10}$ n NaOH to pH = 7.0±0.05 ahead of chromatography. By evaporation, a volume of 1000 ml each was reduced to 3.0 ml. Distilled water served as elution-fluid. The flow-rate of the column was 0.4 cm/min, which equals $1\,cm^3/min$. A fraction collector (type LKB 2112 Redirac) collected fractions of equal volume.

Carbon was analysed by using a Beckman TOC-Analyzer (Mod. 915 B). MRP determinations followed German Standard Methods by using an automatic analysing system. 2% ascorbic acid served as reducting agent.

UV-radiation was performed with a mercury low pressure lamp by Original Hanau (type TQ 150).

Prior to evaporation, the metals in their different concentration, all as chlorides, were added. Prior to GPC, possibly occurring particulate matter was separated by membrane-filtration.

Each test was run in triple parallels.

Results

The separation of preconcentrated fulvic acids of the Latschensee by GPC results in a DOC graph with three peaks with a strong majority of apparently macromolecular substances (see Fig. 1). The peak of these substances has a "shoulder", consisting of apparently lower molecular weight compounds, which were not completely separated from the main peak. Dominated by the

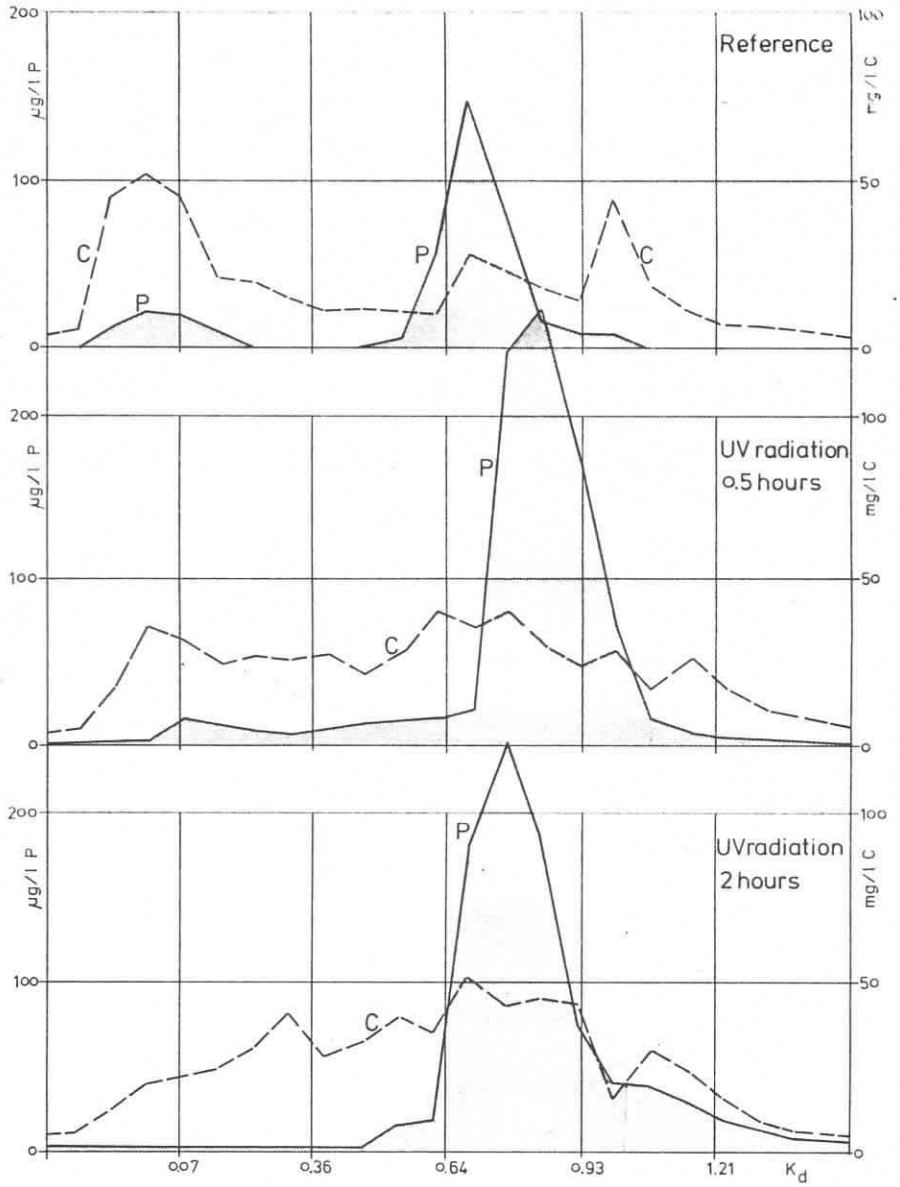

Fig. 1. Gelpermeation chromatography (Sephadex G-25) of pre-concentrated raised peat bog water. Distribution of carbon and MRP. UV-lability of high molecular weight MRP compounds.

lmwt fraction (K_d 0.6 to 1.0), the distribution of MRP-components has two peaks. UV-radiation tests were performed to evaluate geochemical characteristics of associations of fulvic acids and MRP. As expected, UV-radiation (0.5

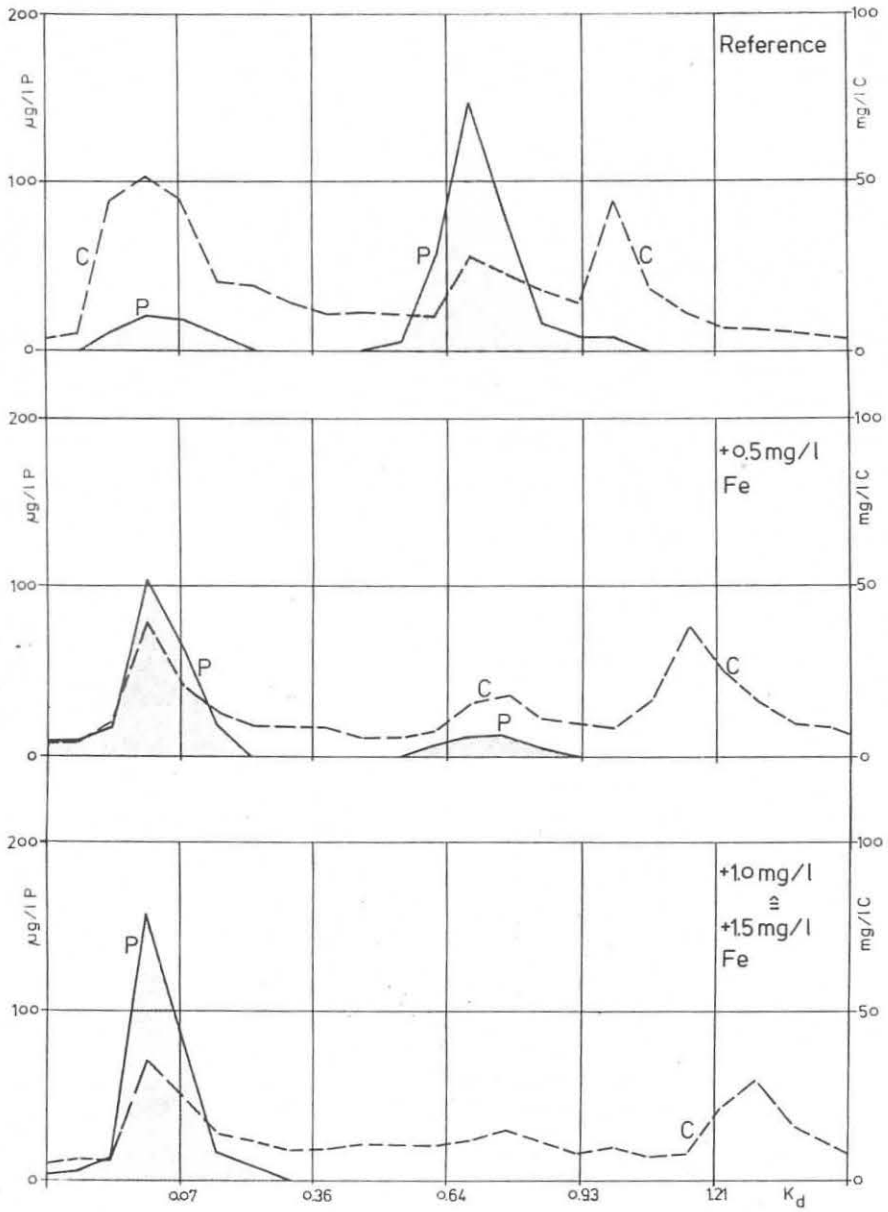

Fig. 2. Additions of iron ions in increasing concentrations.

and 2.0 hrs) destroyed the hmwt fulvic acids and increased lmwt fractions of the DOC. The distribution of distinct mwt fractions becomes more and more unclassified with increasing time of radiation. DOC distribution becomes more or less equalized. MRP in the form of orthophosphate is released from

the hmwt fraction by UV-radiation. After 2 hrs of radiation, the rate of o-PO_4 significantly supercedes the sum of both hmwt and lmwt MRP of the reference sample. It may be assumed that tightly bound phosphates of the hmwt fraction have been set free by UV-radiation as well.

In general, adding metal ions to the water samples reduces the DOC-yield after GPC-separation (see Figs. 2, 3 and 4). For iron and manganese, the loss mechanism might caused by the growth of hydroxide-colloids, which are covered with fulvic acids and are separated by membrane filtration. None such mechanism can be found for the cadmium series. Thus, the following results of metal addition series have to be considered only semiquantitatively.

Addition of iron-ions (Fig. 2)

The addition of iron ions causes a decrease of o-PO_4 and an increase of hmwt MRP at the same time. This process gradually continues up to an iron concentration of 1.5 mg/l. No difference could be observed between the addition of 1.0 and 1.5 mg/l Fe (distinctive representation of these results was therefore relinquished). After adding 5.0 mg/l Fe, the brownish water cleared: iron hydroxide-colloids coprecipitated the organic substances and phosphates. The MRP-maximum of this test's eluate appeared far behind the separation volume of the column, thus, making it impossible to classify the molecular weight fraction of MRP.

Addition of manganese-ions (Fig. 3)

The addition of Mn-ions up to a concentration between 0.5 and 1.0 mg/l Mn causes — as well as the described Fe-series — a decrease of o-PO_4 and a non stoichiometric increase of hmwt MRP. Addition of 1.0 mg/l Mn continues to increase the concentration of hmwt MRP, but releases o-PO_4 simultaneously.

Continued Mn-addition increases o-PO_4 production and leads to a significant decrease of hmwt MRP. The addition of Mn-ions seems to cause two distinctive processes:

— adsorption of o-PO_4 at lower Mn-concentrations and
— a reversion of this process at higher Mn-concentrations.

Addition of cadmium-ions (Fig. 4)

The addition of cadmium-ions causes no increase of hmwt MRP but a release of o-PO_4. To a certain degree, this process seems to be subjected to the concentrations of cadmium added.

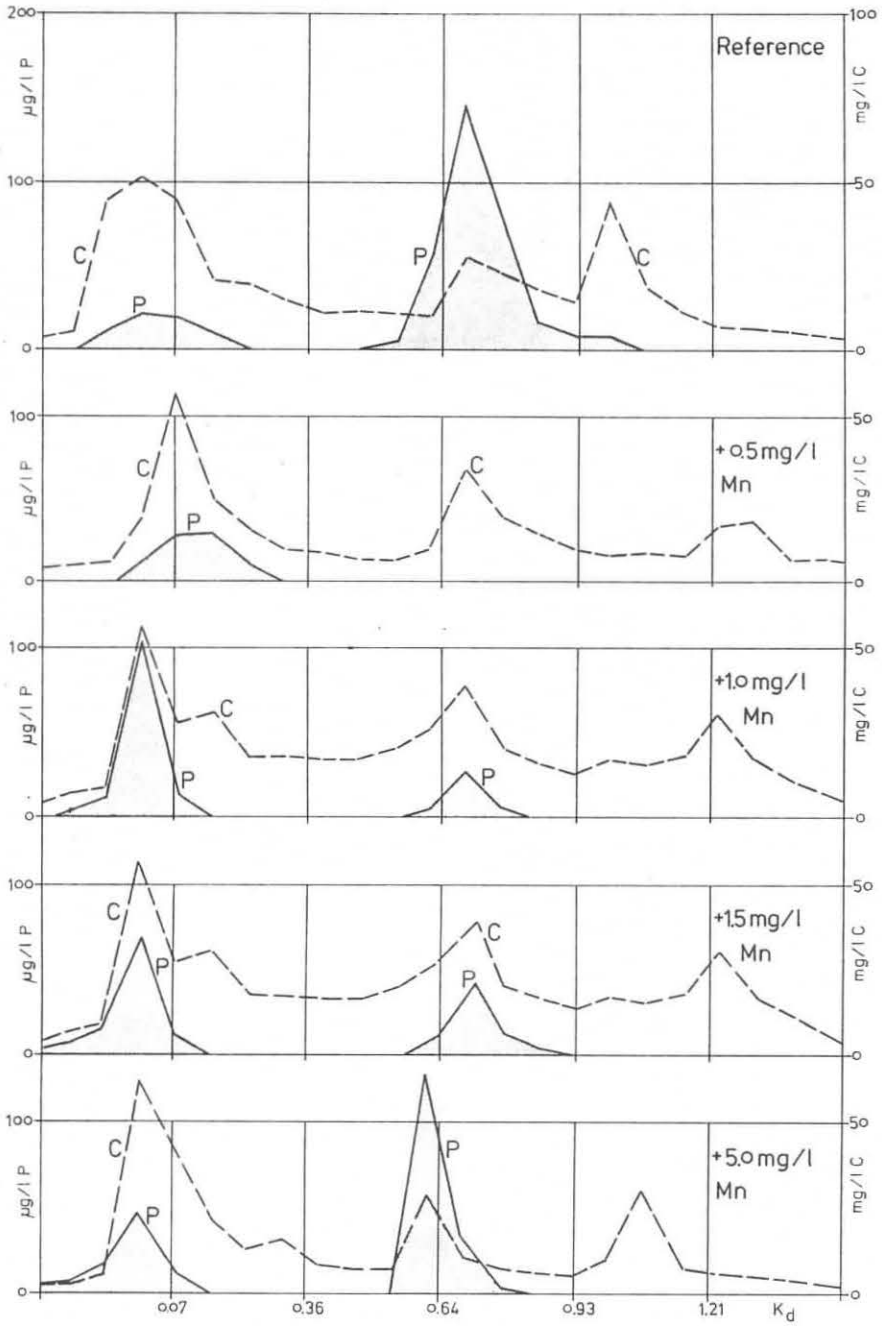

Fig. 3. Additions of manganese ions in increasing concentrations.

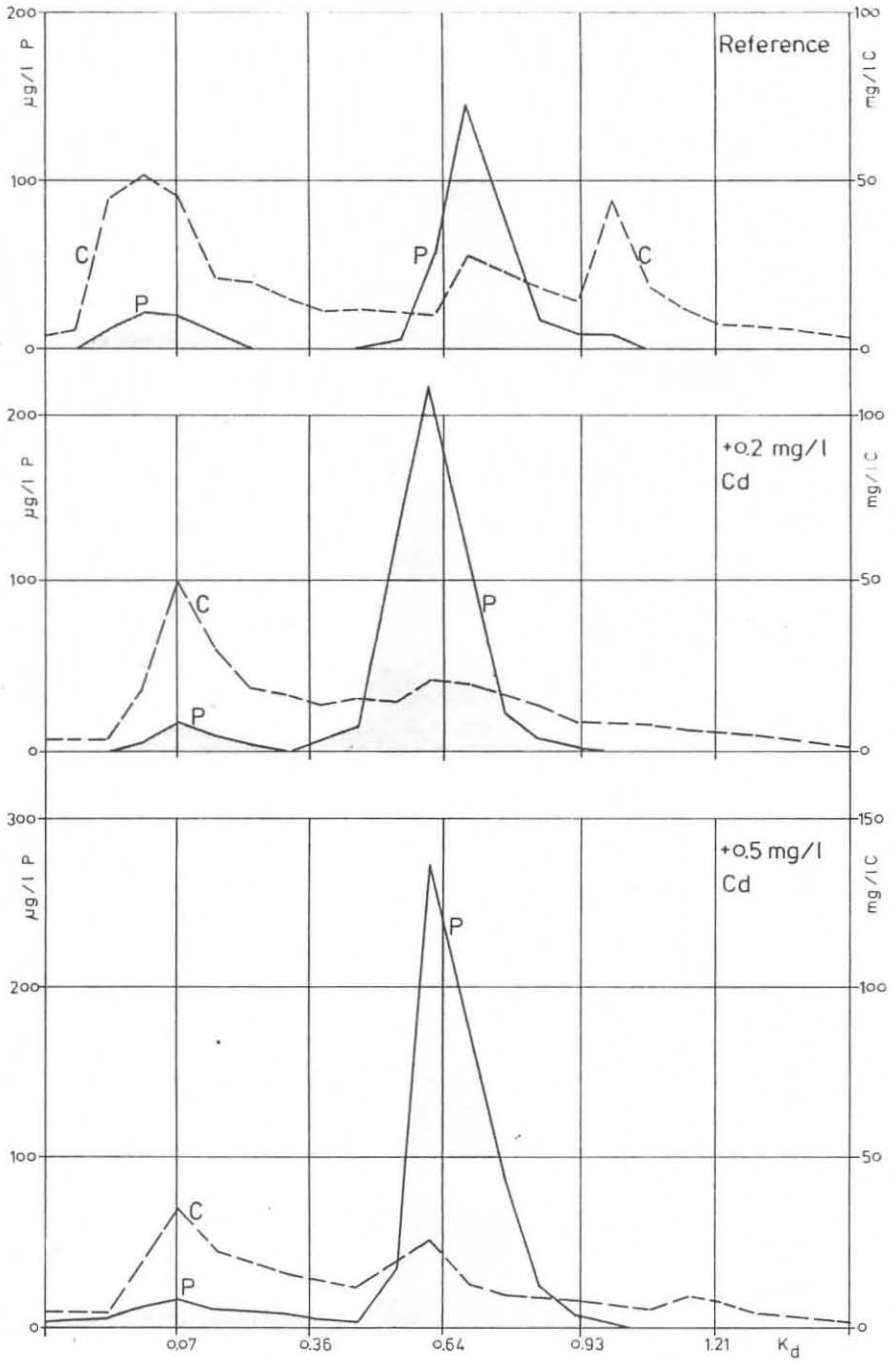

Fig. 4. Additions of cadmium ions in increasing concentrations.

Discussion

In natural waters, the conditions we used in our experiments are present only in the minority of aquatic habitats. Higher concentrations of dissolved iron and manganese can be found in dystrophic waters, especially in the interface of sediment and water or sometimes in the metalimnion as well.

Yet, the pH-values of these waters rarely reach the neutral point, at which our experiments were performed. Higher Cd-concentrations, which might effect the hmwt MRP, are caused by waste (water) pollution and cannot be found under natural conditions. However, some geochemical characteristics of fulvic acids/MRP associations were evaluated in our model tests.

To avoid discussions on the utility of GPC fractionations to assess molecular weights of dissolved organic water constituents (compare Proceedings of the 1st international meeting of the IHSS at Estes Park, Colorado, Aug. 1983), we state that we applied GPC only to distinguish between hmwt and lmwt MRP. No more or less exact molecular weight determination was intended.

Our experiments described above may lead to the following conclusions.

I) To a certain degree, the addition of Fe and Mn causes humic material to sorb phosphate in significant quantities, probably by colloidal adsorption. Colloids consisting of metal hydroxides, humic substances and phosphates have been proven already by Ohle (1935) employing a filtration technique and — to give a more recent example — by Jackson & Hecky (1980) who used infra red analysis.

Humic material with added Cd or without any added metal does not sorb phosphate significantly. The small amount of hmwt MRP in the reference sample may have been produced in the course of preparing the sample, especially during the concentrating step, thus causing iron and manganese of the original water to react in the way described above.

II) Most of the sorbed phosphorus is released as soluble reactive phosphorus by acid in the reagents (cf. Kalle, 1935; Downes & Paerl, 1978; Steinberg & Schrimpf, 1980) or by molybdate-enhanced hydrolysis (cf. Tarapchak, 1983), both used for reactive phosphate determinations.

These results can be interpreted on the basis of the work of Tipping & Higgins (1982) and Francko & Heath (1979, 1982). Under experimental conditions, Fe and Mn, but not Cd, may precipitate as hydroxide colloids that incorporate phosphate, as well as humic substances, and do not coagulate because of steric stabilisation at high adsorption density (Tipping & Higgins, 1982). By dissolving the hydroxides, acidification would reduce phosphate adsorption. If the high molecular weight humic substances/phosphorus complexes described by Francko & Heath are similar to these postulated colloids, UV-light disrupts the phenolic groups of the humic substances in the

colloids, thereby reducing the affinity of Fe or Mn toward phosphate. Phosphates are released subsequently. To have a basis of comparison, reference samples (without metal additions) were radiated by UV-light over different periods of time. As a result, the hmwt MRP components were completely destroyed after 2 hrs at the latest. UV-radiation with it's ecophysiological importance for water layers near the surface on clear sunny days (FRANCKO & HEATH, 1979), has two effects:

1) orthophosphate is released, as described, and is easily taken up by microorganisms in the water.
2) The UV-induced cleavage of fulvic acids increases their biodegradability (STROME & MILLER, 1978).

According to studies of FRANCKO & HEATH (1979), hmwt MRP complexes are refractory to enzymatic hydrolysis. There may be steric inhibition of the enzymes by the phosphorus-metal hydroxide complexes or inactivation of hydrolytic enzymes by dissolved humic substances, as described by BAXTER & CAREY (1982). Furthermore, FRANCKO & HEATH (1982) as well as COLLIENNE (1983) suggested that orthophosphate sorbed to ferric iron/dissolved humic substances complexes may be released by another mechanism: the UV-induced photoreduction of ferric iron to the ferrous state.

As a third relevant result we found that

III) higher additions of Mn and each addition of cadmium caused an increase of o-PO_4. It is imaginable, that these metals, starting at a certain threshold-concentration (Mn), lead to a catalytic release of phosphate. A similar process for molybdenium was described by TARAPCHAK (1983) when analyzing phosphate in limnic samples. According to TARAPCHAK, the mere presence of Mo at neutral pH-value caused a significant release of o-PO_4 from compounds of higher molecular weight. An adequate process might be stimulated by Cd and higher Mn-concentrations. The immediate proof of the catalytic character of these metals is still missing, as well as possible reaction mechanisms. Even with Mo, these mechanisms are not yet satisfactorily described.

Besides the mentioned release mechanisms of phosphate from fulvic acid/MRP associations, OHLE described in 1937 one another mechanism. At alkaline conditions in the water, a great moiety of o-PO_4 is released, as the colloid becomes electronegative and repels the phosphate ion, which also is negatively charged. By this mechanism, 100 % of a certain amount of PO_4-ions may be found in a membrane filtrate, thus, being released. At acidic conditions, only 35 % of the PO_4-ions can be detected in the filtrate.

This electrostatic mechanism was probably not responsible for the phosphate release in our test series, as alkaline conditions were not present.

Zusammenfassung

Organische Wasserinhaltsstoffe aus einem Hochmoorsee und daran assoziierte Molybdänblau-reaktive Phosphate wurden über Sephadex G-25 Gelpermeationschromatographie aufgetrennt. Es ergaben sich eine dreigipflige Verteilung für den gelösten organischen Kohlenstoff und eine zweigipflige für die reaktiven Phosphate. Die reaktiven Phosphate umfaßten eine niedermolekulare (lmwt) und eine hochmolekulare (hmwt) Fraktion. Die hmwt Fraktion erwies sich gegenüber UV-Strahlung als labil.

Der Zusatz von Eisen-Ionen bis zu 1,5 mg/l Fe und von geringen Mengen an Mangan-Ionen führte zu einer Abnahme der lmwt Phosphat-Fraktion bei gleichzeitiger Zunahme der hochmolekularen Phosphat-Fraktion. Als Mechanismus wird die Bildung von Kolloiden aus Metall(oxid)-Hydroxiden mit adsorbierten Humussubstanzen und Phosphaten vermutet. Höhere Zugaben von Mangan-Ionen und jede Zugabe von Cadmium-Ionen erbrachte eine Verminderung der hmwt Phosphate und simultan eine Zunahme der lmwt Phosphate (im wesentlichen Orthophosphat). Das letztgenannte Phänomen wird als katalytische Spaltung der hmwt Phosphate durch die zugefügten Metalle angesehen, wie sie bereits für Molybdän beschrieben wurde. Welche weiteren Metalle zur katalytischen Spaltung von hmwt Phosphaten befähigt sind, muß zukünftigen Studien vorbehalten bleiben.

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Addresses of the authors:

Dr. CHRISTIAN STEINBERG, c/o Bayer. Landesamt für Wasserwirtschaft, Lazarettstr. 67, D-8000 Munich 19.

G. FRIEDRICH BALTES, Brunellenweg 29, D-8000 Munich 21, Fed. Rep. Germany.